

- (34) McWhorter WP, Mayer WJ. Black/white differences in type of initial breast cancer treatment and implication for survival. *Am J Public Health* 1987;77:1515-7.
- (35) Diehr P, Yergan J, Chu J, Feigl P, Glaefke G, Moe R, et al. Treatment modality and quality differences for black and white breast-cancer patients treated in community hospitals. *Med Care* 1989;27:942-58.
- (36) Whittle J, Conigliaro J, Good CB, Lofgren RP. Racial differences in the use of invasive cardiovascular procedures in the Department of Veterans Affairs medical system. *N Engl J Med* 1993;329:621-7.
- (37) Mandelblatt J, Andrews H, Kao R, Wallace R, Kerner J. The late-stage diagnosis of colorectal cancer: demographic and socioeconomic factors. *Am J Public Health* 1996;86:1794-7.
- (38) Liff JM, Chow WH, Greenberg RS. Rural-urban differences in stage at diagnosis. Possible relationship to cancer screening. *Cancer* 1991;67:1454-9.
- (39) Weaver P, Harrison B, Eskander G, Jahan MS, Tanzo V, Williams W, et al. Colon cancer in blacks: a disease with a worsening prognosis. *J Natl Med Assoc* 1991;83:133-6.
- (40) Tejeda HA, Green SB, Trimble EL, Ford L, High JL, Ungerleider RS, et al. Representation of African-Americans, Hispanics, and whites in National Cancer Institute cancer treatment trials. *J Natl Cancer Inst* 1996;88:812-6.
- (41) Watanabe ME. "Amid criticism, NCI tries to boost minority clinical-trial recruitment." *The Scientist* 1996;10:7 p. 1, 4-5, April 1.
- (42) Brown KS. "Scientists, African-American clergy join forces for trial recruitment." *The Scientist* 1997;11:4 p. 1, 10, February 17.
- (43) Macdonald JS. Adjuvant therapy for colon cancer. *CA Cancer J Clin* 1997;47:243-56.

NOTES

Editor's note: SEER is a set of geographically defined, population-based, central cancer registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Registry data are submitted electronically without personal identifiers to the NCI on a biannual basis, and the NCI makes the data available to the public for scientific research.

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$$\textcircled{1} - 7 \times 7 \times 7 \times 0.5 \approx 200 \text{ mm}^3$$

DT-Diaphorase Expression and Tumor Cell Sensitivity to 17-Allylamino,17-demethoxygeldanamycin, an Inhibitor of Heat Shock Protein 90

Lloyd R. Kelland, Swee Y. Sharp, Paul M. Rogers, Timothy G. Myers, Paul Workman

$\textcircled{4}$ 2 human CC lines

Background: To our knowledge, 17-allylamino,17-demethoxygeldanamycin (17AAG) is the first inhibitor of heat shock protein 90 (Hsp90) to enter a phase I clinical trial in cancer. Inhibition of Hsp90, a chaperone protein (a protein that helps other proteins avoid misfolding pathways that produce inactive or aggregated states), leads to depletion of important oncogenic proteins, including Raf-1 and mutant p53 (also known as TP53). Given its ansamycin benzoquinone structure, we questioned whether the antitumor activity of 17AAG was affected by expression of the NQO1 gene, which encodes the quinone-metabolizing enzyme DT-diaphorase. **Methods:** The antitumor activity of 17AAG and other Hsp90 inhibitors was determined by use of a sulforhodamine B-based cell growth inhibition assay in culture and by the arrest of xenograft tumor growth in nude mice. DT-diaphorase activity was determined by use of a spectrophotometric assay, and protein expression was determined by means of western immunoblotting. **Results:** In two independent *in vitro* human tumor cell panels, we observed a positive relationship between DT-diaphorase expression level and growth inhibition by 17AAG. Stable, high-level expression of the active NQO1 gene transfected into the DT-diaphorase-deficient (by NQO1 mutation) BE human colon carcinoma cell line resulted in a 32-fold increase in 17AAG growth-inhibition activity. Increased sensitivity to 17AAG in the transfected cell line was also confirmed in xenografts. The extent of depletion of Raf-1 and mutant p53 protein con-

firmed that the Hsp90 inhibition mechanism was maintained in cells with high and low levels of DT-diaphorase. 17AAG was shown to be a substrate for purified human DT-diaphorase. **Conclusion:** These results suggest that the antitumor activity and possibly the toxicologic properties of 17AAG in humans may be influenced by the expression of DT-diaphorase. Careful monitoring for NQO1 polymorphism and the level of tumor DT-diaphorase activity is therefore recommended in clinical trials with 17AAG. [*J Natl Cancer Inst* 1999;91:1940-9]

Benzoquinone ansamycins, such as herbimycin and geldanamycin (Fig. 1), exhibit anticancer activity by binding to heat shock protein 90 (Hsp90), a molecular chaperone, and its homologue GRP94 (1,2). In this interaction, geldanamycin competes with adenosine triphosphate at the N-terminal-binding site

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of Hsp90 (3). The interaction results in the lysosome-mediated degradation of several important oncogenic proteins, including Raf-1, c-ErbB2, and mutant (but not wild-type) p53 (also known as TP53) (4-6). Clearly, this molecular profile offers considerable potential for antitumor activity. However, both herbimycin and geldanamycin have limitations as drug candidates because of poor stability and hepatotoxicity (7). This has resulted in efforts to discover improved synthetic analogues (8).

One such compound, the 17-allylamino,17-demethoxy analogue of geldanamycin (17-allylamino,17-demethoxygeldanamycin: 17AAG) (Fig. 1), has also been shown to bind to Hsp90 (9). Although, in rodent and dog toxicology studies, 17AAG retains some of geldanamycin's toxicity in the liver, gallbladder, and kidney [(10) and National Cancer Institute (NCI) drug data file on 17AAG] it has a better therapeutic index. For example, 17AAG exerts antitumor activity against some human melanoma xenografts at nontoxic doses [NCI drug data file on 17AAG and (11)]. Preclinical pharmacokinetic studies show that pharmacologically active concentrations can be achieved in plasma and tissues [NCI drug data file on 17AAG and (12)] and that the major liver microsomal metabolite (shown in Fig. 1) is 17-amino,17-demethoxygeldanamycin (13). In view of its novel mechanism of action and its good therapeutic index, 17AAG has now entered phase I clinical trials as first-in-class Hsp90 inhibitor under the auspices of the U.S. NCI and the U.K. Cancer Research Campaign (CRC). Recently, the structurally distinct macrocyclic antifungal compound radicicol (Fig. 1) has been shown to bind to Hsp90 and inhibit its activity (14-17).

DT-diaphorase, an obligate two-electron-reducing enzyme [reduced nicotinamide-adenine dinucleotide (phosphate):quinone oxidoreductase; EC 1.6.99.2], catalyzes the reduction of various quinones (18). As a result, cells rich in DT-diaphorase are especially sensitive to quinone-containing bioreductive anticancer agents, such as mitomycin C and the indoloquinone EO9, which act as prodrugs for activation to toxic forms by DT-diaphorase (19-21). Some tumor types (notably, colon and non-small-cell lung cancers) have been shown to contain rela-

tively high levels of DT-diaphorase (22-26). Thus, these cancers may be particularly suitable for treatments that use a DT-diaphorase prodrug approach. Although previous studies (27) have shown that geldanamycin is a substrate for DT-diaphorase, a cell line derived from human colorectal cancer and expressing DT-diaphorase did not appear to be particularly sensitive to geldanamycin. However, it is not known whether cells expressing high levels of DT-diaphorase show altered sensitivity to 17AAG.

The primary aim of this study was to investigate whether DT-diaphorase activity has a role in the sensitivity of human tumor cells to 17AAG. Initially, sensitivity to 17AAG was determined by use of the CRC/Institute of Cancer Research (ICR) panel of 15 human colorectal and 11 ovarian carcinoma cell lines, including some resistant to classical agents. Comparative data were obtained in selected lines for the 17-amino metabolite and the additional Hsp90-binding agents geldanamycin and radicicol. The correlation between sensitivity and DT-diaphorase activity seen in a subset of the CRC/ICR panel (selected to span the range of sensitivity to 17AAG) was then examined and confirmed with data from the NCI panel of 60 human tumor cell lines (28). This led to the hypothesis that high DT-diaphorase expression was a major factor in determining cellular sensitivity to 17AAG but not to geldanamycin or radicicol. To provide further conclusive data, sensitivity to 17AAG was determined in a newly established isogenic pair of cell lines that differ only in the expression of the active NQO1 gene. This pair is composed of the human colon BE line [which contains a disabling point mutation in the NQO1 gene encoding DT-diaphorase (29)] and a subline stably transfected with the NQO1 gene and expressing high levels of functional DT-diaphorase. Finally, evidence that the Hsp90 inhibitory mechanism was retained by 17AAG in colon cell lines expressing high and low levels of DT-diaphorase was obtained by immunoblot analysis of Raf-1, mutant p53, Hsp70, and Hsp90 proteins. The results suggest that determination of patients' NQO1 genotype and of tumor DT-diaphorase activity should be included in the clinical evaluation of 17AAG because variations in these characteristics could affect the toxicity and efficacy of the drug.

MATERIALS AND METHODS

Cell Lines

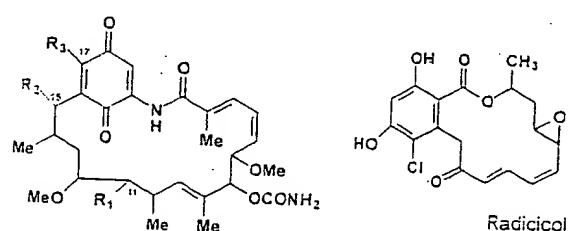
We used panels of human colon and ovarian cell lines. We obtained cell lines from commercial cell culture collections or derived them in-house as described previously (30). In some cases, we used sublines derived from a particular parent line with acquired drug resistance to cisplatin (CH1cisR and A2780cisR ovarian lines) or to doxorubicin (CH1doxR and an SKOV-3 subline stably overexpressing the multidrug-resistance protein MRP1) (30-32). All lines were grown as monolayers in Dulbecco's modified Eagle medium containing 10% fetal calf serum, 2 mM glutamine, and 0.5 µg/mL hydrocortisone in 6% CO₂/94% air. All lines were free of *Mycoplasma* contamination.

Drugs and Chemicals

Geldanamycin, 17AAG, and 17-amino,17-demethoxygeldanamycin were supplied by E. Sausville (NCI). The remaining drugs (herbimycin, radicicol, streptozotocin, and dicoumarol) and chemicals were obtained from Sigma Chemical Co. (Poole, U.K.).

Growth Inhibition Studies

We used the sulforhodamine B assay as described previously (30-32) for growth inhibition studies. Briefly, we seeded tumor cells into 96-well microtiter plates, allowed the cells to attach overnight, and then added the drug to quadru-



	R ₁	R ₂	R ₃
Geldanamycin	OH	H	OMe
17AAG	OH	H	CH ₂ =CHCH ₂ NH
17-amino	OH	H	NH ₂
Herbimycin	OMe	OMe	H

Fig. 1. Chemical structures of geldanamycin, 17-allylamino,17-demethoxygeldanamycin (17AAG), 17-amino,17-demethoxygeldanamycin (17-amino), radicicol, and herbimycin.

plicate wells as indicated. Unless otherwise indicated, we exposed cells to a drug for 4 days. Thereafter, the cell number in treated versus control wells was estimated after treatment with 10% trichloroacetic acid and staining with 0.4% sulforhodamine B in 1% acetic acid. The IC_{50} was calculated as the drug concentration that inhibits cell growth by 50% compared with control growth.

Stable Transfection of the NQO1 Gene Into the BE Human Colon Carcinoma Cell Line

BE cells contain a point mutation in the NQO1 gene and thus have no functional DT-diaphorase enzyme activity (29). We used the bicistronic expression vector pEFIRE5-P (33) to express the NQO1 gene in BE cells. Lipofectamine (Life Technologies, Inc. [GIBCO BRL], Gaithersburg, MD) for transfection, and puromycin (0.5 μ g/mL) for selection. Resulting clones were screened for DT-diaphorase enzyme activity or protein by an enzyme assay or immunoblotting, respectively (see below). Full details of the vector construction and the biologic properties of the stable transfectants will be published elsewhere (Sharp SY, Kelland LR, Valenti MR, Branton LA, Hobbs S, Workman P: unpublished results). The stable transfectants, designated BE-F397 clone 2 and BE-F397 clone 5, were used in these studies.

DT-Diaphorase Assay

To determine whether 17AAG was a good substrate for DT-diaphorase, we used the standard cytochrome *c* assay, as described previously for the bioreductive indoloquinone EO9 (34) and geldanamycin (27), but replaced menadione with 17AAG as the substrate and intermediate electron acceptor. We assayed extracts of the human colon cell line HT29 or purified human DT-diaphorase protein (from J. Skelly, ICR). For preparation of cell extracts, 2×10^7 cells were trypsinized, washed twice in ice-cold phosphate-buffered saline (PBS), and centrifuged (MSE Centaur I; 1100 rpm for 5 minutes at room temperature). The cell pellet then was resuspended in 0.5–1 mL of lysis buffer (PBS containing 1% Triton X-114 and 500 μ M phenylmethylsulfonyl fluoride) and left on ice for 30 minutes. After centrifugation (MSE Microcentrifuge; 12 000 rpm for 5 minutes at room temperature), the supernatant was used for protein determination and the enzyme assay. Results obtained for 17AAG were compared with those for geldanamycin, EO9, and streptonigrin, an excellent substrate for DT-diaphorase (35). For all drugs, the difference in reduction of the menadione substrate in the absence and presence of dicoumarol (100 μ M), a standard inhibitor of DT-diaphorase, was determined (27).

Immunoblotting

This analysis was performed as described previously (30–32). Briefly, 5×10^6 cells were trypsinized, washed with PBS, and lysed in 100 μ L of lysis buffer at 4°C for 1 hour. Lysis buffer contained 10 mL of 150 mM NaCl–50 mM Tris–HCl (pH 7.5), 500 μ L of 20 mM phenylmethylsulfonyl fluoride, 2 μ L of aprotinin (10 mg/mL, stock solution), 2 μ L of leupeptin (10 mg/mL, stock solution), 100 μ L of 10 mM sodium orthovanadate, 100 μ L of Nonidet P-40, and 100 μ L of 20% sodium dodecyl sulfate (SDS). Lysates were centrifuged (MSE Microcentrifuge; 12 000 rpm for 15 minutes at 4°C), and the resulting protein extracts were separated (50 μ g/lane) by SDS–polyacrylamide gel electrophoresis and electroblotted to nitrocellulose filters. Antibodies to Hsp90 and Hsp70 were obtained from StressGen (Victoria, Canada), and antibodies to Raf-1 and p53 (DO1) were from Santa Cruz Biotechnology (Santa Cruz, CA). A monoclonal antibody to the rat DT-diaphorase (which cross-reacts with human diaphorase) was supplied by R. Knox (previously at CRC/ICR, now at Enzacta Ltd., Salisbury, U.K.). Antibody binding was identified with horseradish peroxidase-labeled secondary antibodies combined with enhanced chemiluminescence reagents (Amersham, Buckinghamshire, U.K.) and autoradiography.

In Vivo Effects

BE vector control cells and BE-F397 clone 2 cells were established as subcutaneous xenografts by injection of 5×10^6 cells into the flanks of adult female athymic nude (nu/nu) mice. The antitumor effect of 17AAG was determined in mice bearing comparably sized tumors (6–8 mm in diameter) derived from these cells. Animals were randomly assigned to receive vehicle alone (five or six mice) or 17AAG (five animals; dose schedule = 80 mg/kg per day in 10% dimethyl sulfoxide and 90% egg phospholipid by intraperitoneal injection on days 1–4 and days 7–11). Before this clinical formulation was available, 17AAG was administered to mice bearing HT29 xenografts in 10% dimethyl sulfoxide–0.05% Tween 20–90% NaCl, with a dose schedule of 80 mg/kg per day on days 0–3 and

days 6–10. This dose and schedule were derived from previously performed experiments [NCI drug data file on 17AAG and (11)].

Tumor size was determined twice weekly by caliper measurements, and tumor volumes were calculated (volume = $[a \times b^2 \times \pi]/6$, where *a* and *b* are orthogonal tumor diameters). Tumor volumes were then expressed as a percentage of the volume at the start of treatment (relative tumor volume). The effect of the drug was determined by the growth delay, i.e., the difference in days required for the volume of tumors in control and treated animals to double. All procedures involving animals were performed within the guidelines set out by the Institute's Animal Ethics Committee and the United Kingdom Coordinating Committee for Cancer Research's *ad hoc* Committee on the Welfare of Animals in Experimental Neoplasia (36).

Statistical Analyses

Where indicated, errors are presented as standard deviation ($n \geq 3$). Correlation tests and linear regression analyses were computed with SAS JMP (SAS Institute, Cary, NC). We assessed correlations with a Spearman calcul for the CRC/ICR panel and with a Pearson calculation for the NCI panel. Although the Spearman statistic is technically more robust, the Pearson statistic was used for correlations in the NCI panel for historic continuity. The likelihood test for linear model comparison was performed with S-Plus (Mathsoft, Seattle, WA). All *P* values are two-sided.

RESULTS

In Vitro Growth Inhibition

The *in vitro* growth inhibition properties of geldanamycin, 17AAG, and radicicol against panels of human colon (15 lines) and ovarian (11 lines) carcinoma cell lines are shown in Table 1, A. The IC_{50} value for 17-amino-17-demethoxygeldanamycin, the major metabolite of 17AAG, is also included for some lines. In most cell lines, all four compounds potently inhibited growth, with IC_{50} values of less than 2.5 μ M. Notably, one ovarian cell line (the 41M line) was relatively resistant ($IC_{50} > 2.5 \mu$ M) to all four Hsp90-interactive compounds. On average, geldanamycin was the most potent agent (mean $IC_{50} = 50.1$ nM), with similar values obtained for 17-amino-17-demethoxygeldanamycin (mean $IC_{50} = 47$ nM in a subset of nine cell lines). 17AAG showed intermediate potency (mean $IC_{50} = 220.4$ nM), and the least potent agent was radicicol (mean $IC_{50} = 587.4$ nM).

Bar graphs showing the IC_{50} values (Fig. 2) reveal some interesting differences in the patterns of response for geldanamycin, 17AAG, and radicicol. Notably, some cell lines (e.g., BE and LoVo colon cells) are relatively resistant to 17AAG but not to geldanamycin (or radicicol). In contrast, the colon cell lines LS174T and KM12 were relatively resistant to geldanamycin but not to 17AAG. We have compared patterns of response for 25 cell lines (excluding 41M because this line was resistant to all compounds) by use of the Spearman analysis. Positive, but not statistically significant, correlations were observed between geldanamycin and radicicol ($r = .36$; $P = .08$) and between geldanamycin and 17AAG ($r = .33$; $P = .11$). There was, however, no correlation between 17AAG and radicicol ($r = -.08$; $P = .72$). Results indicate relatively distinct patterns of response for the three compounds. 17-Amino-17-demethoxygeldanamycin was studied in only a few lines in the panel. With the exception of LS174T colon cells, which are relatively resistant to geldanamycin and more sensitive to the 17-amino metabolite, the two compounds behaved similarly across the panel.

Activity in Acquired Anticancer Drug-Resistant Cell Lines

The *in vitro* potencies of geldanamycin, 17AAG, and radicicol have also been evaluated in various anticancer drug-resistant

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Table 1. *In vitro* human tumor cell growth inhibition by 17AAG and other heat shock protein inhibitors

A. Summary of growth inhibition (drug concentrations that inhibit growth by 50% (IC_{50}) of geldanamycin, 17AAG, radicicol, and 17-amino against the CRC/ICR panel of human colon and ovarian tumor cells*.

Cell line	Geldanamycin	17AAG	Radicicol	17-Amino
Colon				
BE	19.3 ± 3.1	773 ± 30.6	190	18
HT29	46.7 ± 9	8.9 ± 2.9	3100	6.3 ± 1.8
COLO205	3.8	7.2	1400	ND
DLD-1	78	140	290	ND
HCA-7	1.8	72	120	ND
HCC2998	98	78	650	ND
HCT15	83	490	280	ND
HCT116	67	99	240	ND
HT29	11.3	13.5	390	17
KM12	54	9	135	ND
LIM1215	9	77	100	20.5
LS174T	245	78.5	780	33.5
LoVo	21.9	1130	360	42
MAWI	6.2	58	1850	ND
SW620	3.1	68	110	ND
Ovarian				
A2780	11.5	12	115	43
CHI	104.5	1055	325	190
HX62	47	670	2500	ND
IGROV-1	94	92	295	ND
OVCAR-3	5	58	69	ND
OVCAR-4	9.6	295	540	ND
OVCAR-5	88	40	660	ND
OVCAR-8	15.5	67	230	ND
PXN94	84	43	1450	ND
SKOV-3	46	76	395	58
4IM	>2500	5200	2350	>2500

B. Growth inhibitory properties of geldanamycin, 17AAG, and radicicol against anticancer drug-resistant human tumor cell lines*.

Cell line	Geldanamycin	17AAG	Radicicol
A2780	7.1	2.4	115
A2780cisR	9.1	1.9	270
RF	1.3	0.8	2.3
CHI	49	960	325
CH1cisR	17	1220	88
RF	0.35	1.3	0.27
CH1doxR (Pgp)	2500	>2500	565
RF	51	>2.6	1.7
SKOV-3 puro	97.3	46	280
SKOV-3 S2 (MRP)	337	142	280
RF	3.5	3.1	1.0

*17AAG = 17-allylamino,17-demethoxygeldanamycin; 17-Amino = 17-amino,17-demethoxygeldanamycin; CRC = Cancer Research Campaign; ICR = Institute of Cancer Research; MRP = multidrug-resistance protein; Pgp = P-glycoprotein; puro = puromycin; ND = not done; RF = resistance factor; IC_{50} -resistant or -transfected line/parent/vector control line.

†Data are either the mean ± standard deviation (n = 3) or the mean of two determinations.

cell lines. These lines possess acquired resistance to cisplatin (cisR lines) or to doxorubicin through overexpression of P-glycoprotein (doxR line) or of MRP1 (SKOV-3 S2) (Table 1, B). Although little cross-resistance to geldanamycin was observed in the cisplatin-resistant cell lines, geldanamycin was markedly more potent in the P-glycoprotein-overexpressing cell lines and in the MRP1-overexpressing cell lines than in the parent lines, suggesting that geldanamycin is a substrate for these multidrug-

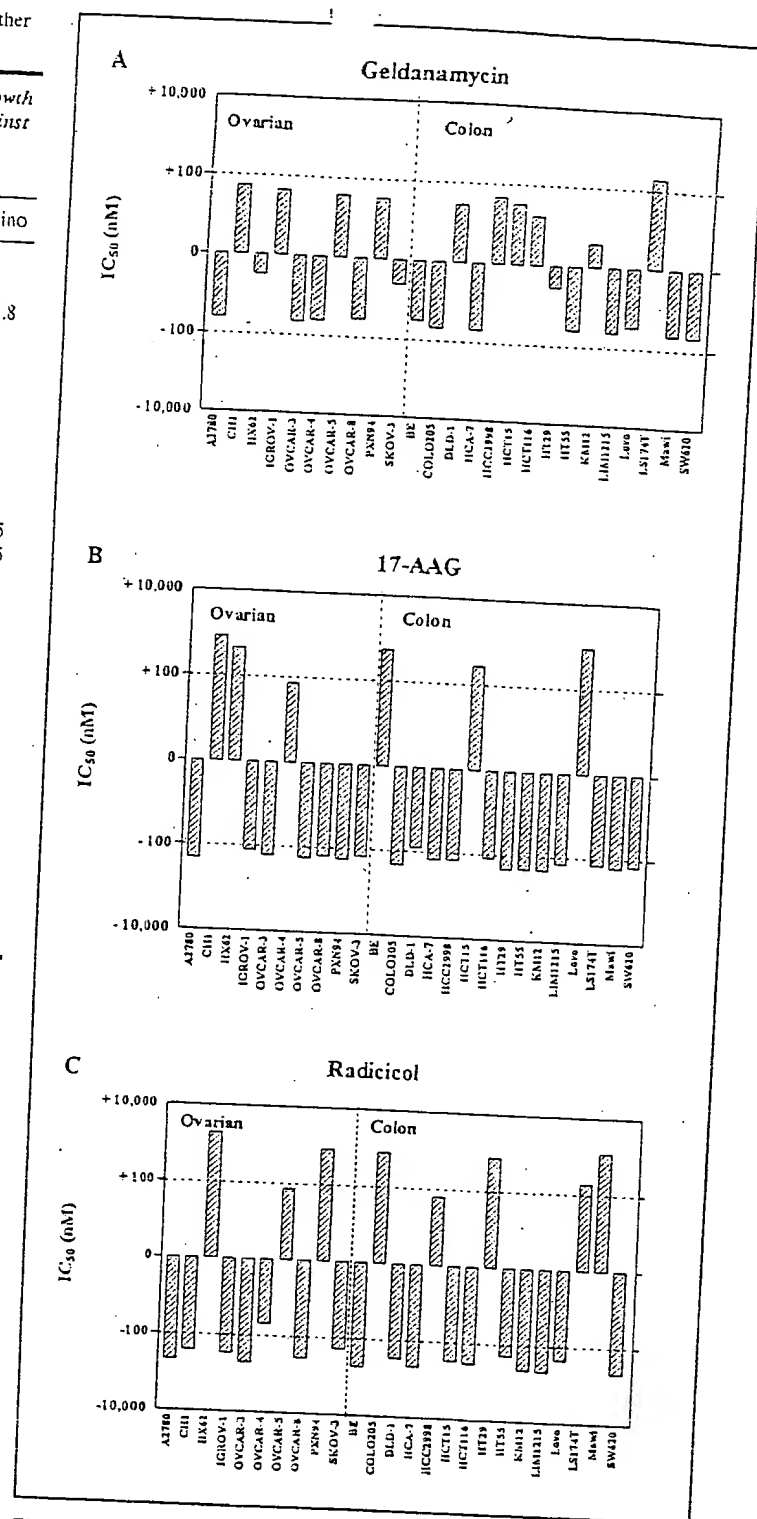


Fig. 2. Patterns of *in vitro* growth inhibition response across 25 human colon or ovarian carcinoma cell lines (as indicated) for geldanamycin (A), 17-allylamino,17-demethoxygeldanamycin (17-AAG) (B), and radicicol (C). Results are displayed as the extent to which the IC_{50} value (mean drug concentrations that inhibit growth by 50% for a 96-hour exposure) for a given cell line is greater or lower than the mean IC_{50} calculated for the entire panel. Values for the mean IC_{50} across the whole panel were as follows: 50.1 nM for geldanamycin, 220.4 nM for 17-AAG, and 587.4 nM for radicicol.

resistant efflux proteins. The picture is rather less clear for 17AAG because the parental CHI ovarian cell line is relatively resistant to 17AAG, although there is at least a 2.5-fold cross-resistance to 17AAG in CHI doxR. The level of cross-resistance

for geldanamycin and 17AAG is similar in the MRP-overexpressing ovarian line. Like geldanamycin, 17AAG retains full activity in the cisplatin-resistant lines.

Growth Inhibition and DT-Diaphorase Enzyme Activity

Because geldanamycin and 17AAG are quinone-based compounds and BE cells have a disabling point mutation in the NQO1 gene (29), the lack of DT-diaphorase activity in these cells could be involved in their surprisingly high relative resistance to 17AAG and low relative resistance to geldanamycin. To explore this possibility, we measured DT-diaphorase enzyme activity and IC_{50} values for geldanamycin, 17AAG, and radicicol in 11 cell lines (selected from those shown in Table 1), with a broad spectrum of responses to these compounds (Fig. 3). A statistically significant negative Spearman correlation was apparent for 17AAG ($r = -.81$; $P = .002$). Cells with marginal DT-diaphorase levels were relatively resistant to 17AAG, but there was no statistically significant correlation between sensitivity to geldanamycin or radicicol and DT-diaphorase levels ($P = .33$ and $.76$, respectively). Thus, we have identified the potential for a causal link between expression of DT-diaphorase and sensitivity to 17AAG, but not geldanamycin, in the CRC/ICR panel of colorectal and ovarian cell lines.

We then repeated this analysis with the NCI panel of 60 cell lines, which are derived from a diverse group of human cancers (28). We have reported previously the DT-diaphorase activities for this panel of cells (23) as the logarithmically transformed values that are normally used for analysis in the NCI panel. We used correlation tests to explore the hypothesis that DT-diaphorase levels could be directly responsible for the sensitivity differences observed among the cell lines. The Pearson correlation coefficient indicated a weak positive relationship between DT-diaphorase expression and sensitivity to 17AAG ($r = .11$). The correlation between DT-diaphorase and geldanamycin was also weak, with possibly a negative trend ($r = -.15$). Neither correlation was statistically significant ($P = .43$ and $.24$, respectively). We then tested the hypothesis that, although DT-diaphorase activity may not predict sensitivity to 17AAG directly, it might explain why some cell lines are more sensitive to 17AAG than to geldanamycin. We tested this hypothesis by comparing the following two linear regression models: 1) 17AAG sensitivity = geldanamycin sensitivity + error, and 2) 17AAG sensitivity = geldanamycin sensitivity + DT-diaphorase activity + error. Because 17AAG and geldanamycin are reasonably well correlated ($r = .50$; $P < .001$), both models fit the data well. However, more important, inclusion of DT-diaphorase caused a statistically significant ($P = .03$) improvement in the fit as measured with a likelihood ratio test (analysis of variance by use of the F statistic). Thus, DT-diaphorase is a statistically significant factor when the sensitivity patterns of 17AAG are compared with those of geldanamycin. Addition of multidrug resistance protein status, as measured functionally by rhodamine efflux, did not improve the above model (data not shown).

Activity in Isogenic BE Colon Cell Lines That Contain or Lack the Active NQO1 Gene

To more directly investigate the role of DT-diaphorase in mediating the cytotoxicity of 17AAG, we stably transfected the BE cell line with the NQO1 gene encoding DT-diaphorase. As shown by immunoblotting, the resulting BE-F397 clone 2 and

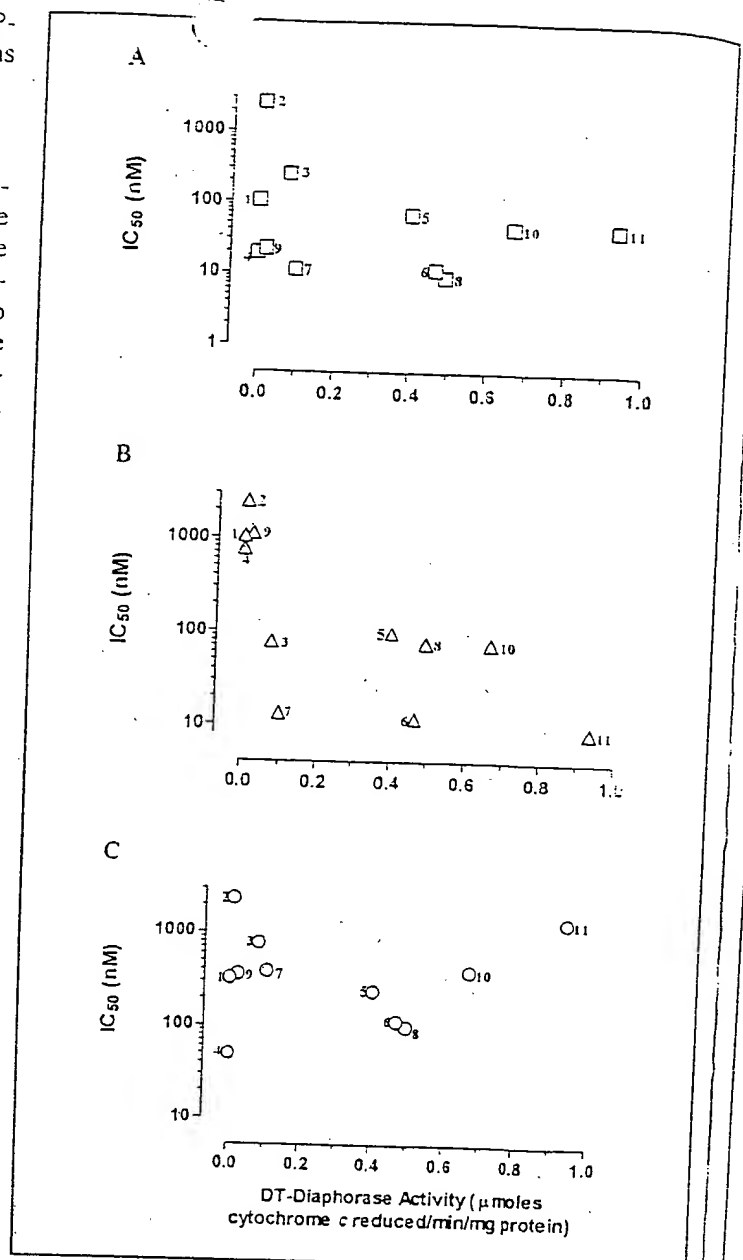


Fig. 3. Relationship between *in vitro* growth inhibition and DT-diaphorase expression for heat shock protein 90 inhibitors. The results are displayed as a plot of IC_{50} (mean drug concentrations that inhibit growth by 50% from a 96-hour exposure) versus DT-diaphorase enzyme activity and DT-diaphorase enzyme activity for geldanamycin (A), 17-allylamino, 17-demethoxygeldanamycin (B), and radicicol (C) in the following 11 human tumor cell lines: 1 = CH1 (ovarian); 2 = 41M (ovarian); 3 = LS174T (colon); 4 = BE (colon); 5 = HCT116 (colon); 6 = A2780 (ovarian); 7 = HT55 (colon); 8 = LIM1215 (colon); 9 = LoVo (colon); 10 = SKOV-3 (ovarian); and 11 = HT29 (colon).

the naturally high DT-diaphorase-containing colon line HT29 possess similar levels of DT-diaphorase protein (unpublished results). Enzyme activity data supported the immunoblotting observations. Values (measured as the dicoumarol-inhibitable reduction of menadione and expressed as micromoles of cytochrome c reduced per minute per milligram of protein) are as follows: BE vector control cells, unmeasurable activity (<0.002); BE-F397 clone 2, 1.4 ± 0.5 ; BE-F397 clone 5, 1.3 ± 0.2 ; and HT29, 0.94 ± 0.2 . This HT29 activity is similar to the activity obtained previously with the same assay (27). Functional validation of the model was provided by the observation

Fig. 4. Heat shock 1 control symbol (17AAG) compared to geldanamycin and radicicol. The symbol indicates the level of DT-diaphorase activity in control/E

that introduction of the DT-diaphorase gene into BE cells substantially enhanced the potency of streptonigrin, an excellent DT-diaphorase substrate and bioreductive agent. The degree of potentiation correlated with DT-diaphorase levels and activity (117-fold potentiation in BE-F397 clone 5 and 142-fold potentiation in BE-F397 clone 2). Further details will be published elsewhere.

Dose-response curves for geldanamycin and 17AAG in BE vector control cells and BE-F397 clone 2 are shown in Fig. 4, A. Although the two lines showed similar sensitivity to geldanamycin, BE vector control cells lacking DT-diaphorase were markedly less sensitive to 17AAG. The degrees of potentiation (in terms of IC_{50} values) for geldanamycin, 17AAG, 17-amino,17-demethoxygeldanamycin, radicicol, and herbimycin observed when DT-diaphorase was introduced into the BE colon cell line are shown in Fig. 4, B. Notably, a 32-fold potentiation

was observed with 17AAG, whereas a less than threefold potentiation was observed for all other compounds evaluated. In a second test of the effect of DT-diaphorase on the growth inhibitory properties of these compounds (Fig. 4, B), HT29 colon cells (naturally high in DT-diaphorase activity) were compared with BE parent cells (no measurable DT-diaphorase activity). Results generally mirrored those results observed with the isogenic-transfected pair of BE lines, with only 17AAG, of the Hsp90 inhibitors tested, showing a marked DT-diaphorase-mediated differential effect (87-fold potentiation). It is of interest in this pair of lines that HT29 cells had a strikingly greater sensitivity to radicicol than did BE cells, an effect not seen with the isogenic BE cell line pair.

Reduction of 17AAG by Purified Human DT-Diaphorase

Having demonstrated a potentially important role for DT-diaphorase in cellular sensitivity to 17AAG, we used a menadi-one substrate replacement assay as described previously (27,34) to determine the ability of this agent, geldanamycin, and 17-amino,17-demethoxygeldanamycin to act as substrates for purified human DT-diaphorase (Table 2). Streptonigrin (35), an excellent substrate for DT-diaphorase, was also included in the comparison. We found that 17AAG was a reasonable substrate for DT-diaphorase, but it is not appreciably better than geldanamycin or 17-amino,17-demethoxygeldanamycin. This is perhaps surprising in view of the cellular data. The DT-diaphorase-mediated reduction rate was similar for all three analogues, each at a substrate concentration of 10 μM . At 50 μM , 17AAG and 17-amino,17-demethoxygeldanamycin gave twofold to threefold higher rates than geldanamycin, and the difference was even greater at 100 μM . Geldanamycin at 100 μM resulted in substrate inhibition, which was not observed with the other two analogues at 100 μM . The latter two concentrations, however, are much higher than the pharmacologically relevant range. It also should be noted that all three of the ansamycin analogues gave reaction rates that were substantially lower than rates observed for streptonigrin (Table 2). With the structurally distinct Hsp90 inhibitor radicicol, which lacks a quinone moiety, no reduction was observed.

Effects of 17AAG on Hsp90, Hsp70, and Oncogenic Proteins

To determine whether the mode of action of 17AAG was the same in cells expressing low and high levels of DT-diaphorase and to guide the choice of molecular pharmacodynamic markers in the imminent clinical trial, we measured the levels of Raf-1, mutant p53, Hsp90, and Hsp70 proteins in vector control cells and transfected BE cells treated with 17AAG (or geldanamycin). Levels of these proteins 6 and 24 hours after the addition of equitoxic (continuous exposure to 5x and 10x IC_{50}) or equimolar (0.15 and 0.3 μM) geldanamycin or 17AAG are shown in Fig. 5. No change in Hsp90 protein levels was observed. A similar marked reduction, especially at 24 hours, was observed for Raf-1 and p53 proteins in the BE vector control cells and BE-F397 clone 2 cells at equitoxic concentrations. By contrast, an increase in Hsp70 levels was observed. For geldanamycin or 17AAG at equimolar concentrations (0.15 or 0.3 μM), no change in any of the four proteins was observed in the BE vector control cells expressing low levels of DT-diaphorase, consistent with their cellular resistance at these concentrations.

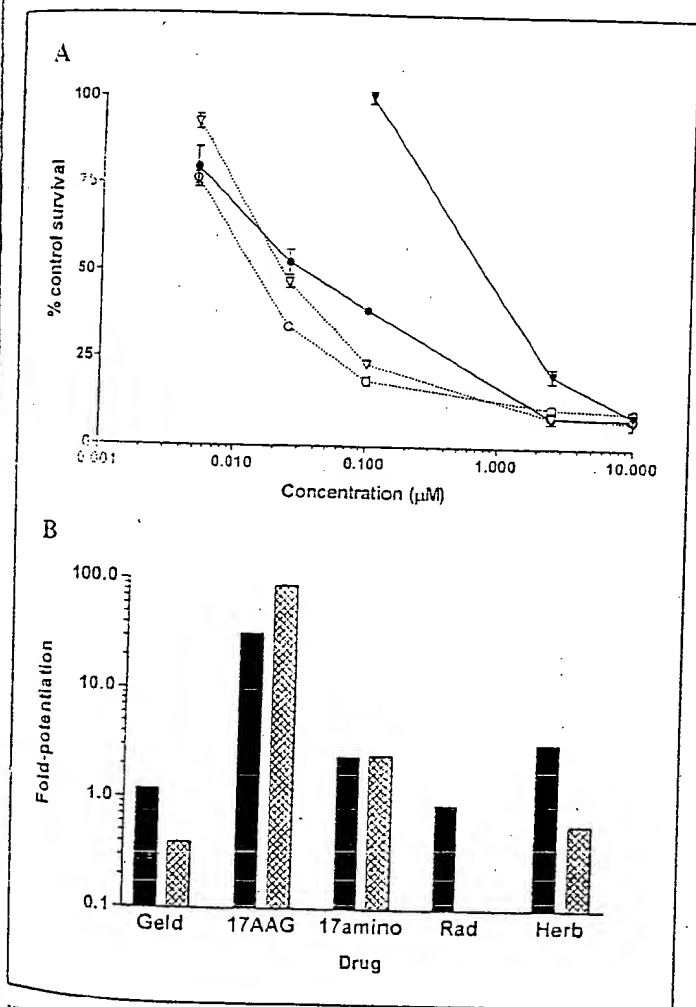


Fig. 4. Effects of DT-diaphorase gene (NQO1) transfection on response to heat shock protein 90 (Hsp90) inhibitors. A) Dose-response curves for BE vector control (∇ , ∇) and BE-F397 clone 2 (\bullet , \circ) for geldanamycin (Geld; open symbols, broken lines) and 17-allylamino,17-demethoxygeldanamycin (17AAG; solid symbols, continuous lines). B) Extent of potentiation in high concentrations that inhibit growth by 50% (IC_{50}) in cells expressing low levels of DT-diaphorase (BE parental or BE vector control line)/ IC_{50} in cells expressing a high level of DT-diaphorase (BE-F397 or HT29 colon line). Solid bars = BE vector control/BE-F397 cells; cross-hatched bars = BE/HT29 cells.

Table 2. Reduction of geldanamycin, 17-allylamino, 17-demethoxygeldanamycin (17AAG), and 17-amino, 17-demethoxygeldanamycin (17-amino) by purified human DT-diaphorase (at 20 $\mu\text{g}/\text{mL}$)*,†

Substrate	Reduction of substrate,* μmol of cytochrome c reduced per minute per mg of protein
Menadione, 10 μM	1188.5 \pm 163.7
Streptonigrin, 50 μM	206.1 \pm 6.0
Streptonigrin, 25 μM	159.1 \pm 9.0
Streptonigrin, 10 μM	176.6 \pm 69.5
Geldanamycin, 100 μM	1.5 \pm 0.8
Geldanamycin, 50 μM	7.2 \pm 3.3
Geldanamycin, 25 μM	7.0 \pm 1.0
Geldanamycin, 10 μM	4.3 \pm 0.6
17AAG, 100 μM	20.5 \pm 2.1
17AAG, 50 μM	15.0 \pm 7.9
17AAG, 25 μM	8.3 \pm 1.3
17AAG, 10 μM	3.6 \pm 0.6
17-Amino, 100 μM	17.6 \pm 5.3
17-Amino, 50 μM	22.8 \pm 3.6
17-Amino, 25 μM	11.7 \pm 4.3
17-Amino, 10 μM	6.8 \pm 3.5
Radicalol	ND

*Values are individual or mean \pm standard deviation ($n = 3$).

†ND = not detectable at all concentrations tested.

In Vivo Effects of 17AAG

We determined the effect of 17AAG on the response of the BE vector control cells and BE-F397 cells when grown subcutaneously as solid tumor xenografts in nude mice. 17AAG was administered at the maximum tolerated dose of 80 mg/kg per day intraperitoneally on days 0–4 and days 7–11, a schedule that is active on sensitive xenografts [NCI drug data file on 17AAG and (11)]. The xenograft tumor grown from the transfected BE-F397 cells (Fig. 6, B) was more sensitive than the BE vector control cells (Fig. 6, A). The growth delays, calculated from the time required to reach twice the treatment volume, were 11.4 days for the BE-F397 xenograft and 5.8 days for the vector control. For the HT29 xenograft (and a similar schedule of 80

mg/kg per day intraperitoneally on days 0–3 and days 6–10), a growth delay of 16.6 days was observed (Fig. 6, C). Experiments (not shown) confirmed that the differences in DT-diaphorase expression seen *in vitro* were maintained in the xenograft (data not shown). Thus, the HT29 line with a naturally high level of DT-diaphorase and also the transfected BE-F397 line were more sensitive *in vivo* than the BE vector control cells that have a low level of DT-diaphorase activity.

DISCUSSION

17AAG is currently entering phase I clinical trial as the first-in-class Hsp90 inhibitor, under the auspices of the NCI and CRC. Treatment with this drug results in the depletion of a number of important oncogenic proteins, including Raf-1, ErbB2, and mutant p53 proteins, from tumor cells (1,4–6,9). In this article, we show that the levels of DT-diaphorase activity in a tumor cell are an important and statistically significant determinant of how well 17AAG will inhibit the growth of that tumor cell. Evidence for this role of DT-diaphorase comes from the following three observations: 1) There was a statistically significant correlation between DT-diaphorase activity and sensitivity to 17AAG for 11 human colon and ovarian cancer cell lines from the CRC/ICR panel. 2) Subsequent interrogation of data from the NCI panel of 60 human tumor cell lines supported the hypothesis that the level of DT-diaphorase activity was a contributory factor in the differences in the sensitivity of tumor cell lines to 17AAG compared with geldanamycin. [In an analogous way, the differences in sensitivity between methotrexate and trimetrexate in the NCI 60 human tumor cell line panel have been explained by differences in the levels of reduced folate carrier protein (37).] 3) Transfection of DT-diaphorase into the BE human colon cancer cell line, thereby creating pairs of isogenic cell lines differing only in DT-diaphorase expression, resulted in a marked increase in 17AAG-induced growth inhibition *in vitro* and an increased response to 17AAG *in vivo*. The degree

Fig. 5. Representative immunoblots for heat shock protein 90 (Hsp90), RAF-1, p53, and Hsp70 (as indicated) in BE vector control or BE clone 2 cells exposed to equitoxic concentrations (5 \times or 10 \times drug concentrations that inhibit growth by 50% [IC_{50}]) of geldanamycin (0.2 and 0.4 μM for 5 \times and 10 \times IC_{50} in BE vector control cells and 0.1 and 0.2 μM for 5 \times and 10 \times IC_{50} in BE-F397 clone 2 cells, respectively) or 17-allylamino, 17-demethoxygeldanamycin (17AAG; 7 and 14 μM for 5 \times and 10 \times IC_{50} in BE vector control cells and 0.15 and 0.3 μM for 5 \times and 10 \times IC_{50} in BE-F397 clone 2 cells, respectively). Two fixed concentrations of 17AAG (0.15 and 0.3 μM) are also shown for RAF-1 in the BE vector control cells. Cells were exposed to drug for 2 hours and harvested 6 and 24 hours after exposure. Lane 1 = 6-hour incubation of untreated cells; lane 2 = 6-hour incubation in geldanamycin (5 \times IC_{50}); lane 3 = 24-hour incubation in geldanamycin (5 \times IC_{50}); lane 4 = 6-hour incubation in geldanamycin (10 \times IC_{50}); lane 5 = 24-hour incubation in geldanamycin (10 \times IC_{50}); lane 6 = 6-hour incubation in 17AAG (5 \times IC_{50}); lane 7 = 24-hour incubation in 17AAG (5 \times IC_{50}); lane 8 = 6-hour incubation in 17AAG (10 \times IC_{50}); lane 9 = 24-hour incubation in 17AAG (10 \times IC_{50}); and lane 10 = 24-hour incubation of untreated cells. Blots for the BE vector control cells and 17AAG are also shown. Lane 11 = 6-hour incubation of untreated cells; lane 12 = 6-hour incubation in 0.15 μM 17AAG; lane 13 = 24-hour incubation in 0.15 μM 17AAG; lane 14 = 6-hour incubation in 0.3 μM 17AAG; lane 15 = 24-hour incubation in 0.3 μM 17AAG; and lane 16 = 24-hour incubation of untreated cells.

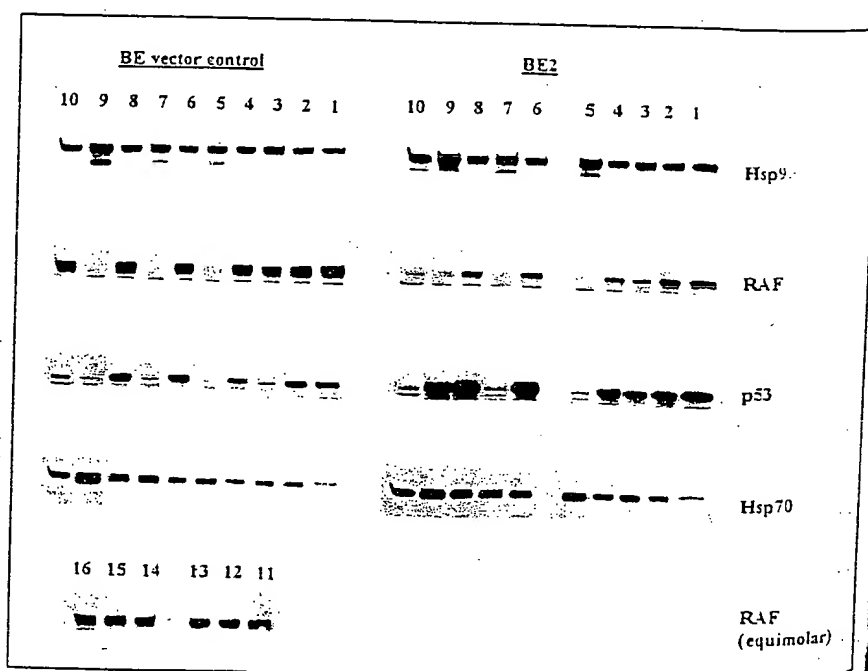


Fig. 6. xenograft tumor growth or HT29 80 mg/kg tumors; control; from five

of *in vivo* express the high transfected the DT cin, res (27). M demeth 17AAG (13) and 17AAG) potency fibrotic diaphorase

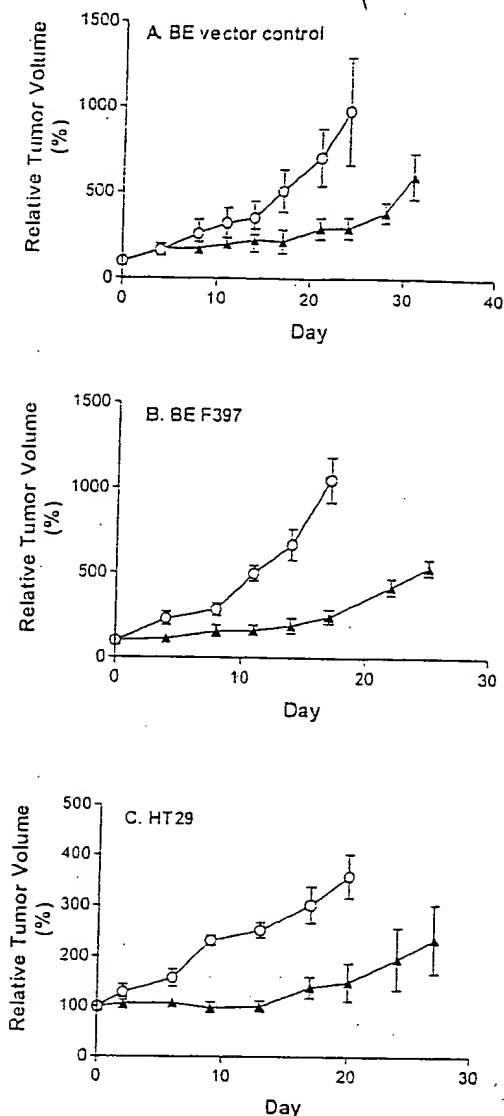


Fig. 6. Effect of DT-diaphorase expression on the response of human tumor xenografts *in vivo* to 17-allylamino,17-demethoxygeldanamycin (17AAG). Tumor growth curves for mice bearing BE vector control (A), BE-F397 clone 2 (B), or HT29 (C) xenografts after treatment with 17AAG. The dosing schedule was 80 mg/kg per day intraperitoneally daily on days 1-4 and days 7-11 for BE tumors and on days 0-3 and days 6-10 for HT29 tumors. \blacktriangle = 17AAG; \circ = control. Data for relative tumor volumes are the means (\pm standard deviation) from five animals.

of *in vitro* growth inhibition correlated with the level of enzyme expression, being 32-fold higher in the transfected cell line with the higher levels of DT-diaphorase and 22-fold higher in the transfected cell line with somewhat lower levels. Of interest, the DT-diaphorase effect was not observed with geldanamycin, results in agreement with data from a nonisogenic pair (27). Moreover, the effect was not seen with 17-amino,17-demethoxygeldanamycin, which was identified as the major 17AAG metabolite in human and mouse hepatic preparations (13) and confirmed as such *in vivo* (NCI drug data file on 17AAG). DT-diaphorase activity also appeared unrelated to the potency of radicicol, the structurally distinct Hsp90-binding antibiotic. Indeed, BE cells that express a low level of DT-diaphorase were almost 10-fold more sensitive to radicicol than

were HT29 cells, which express a naturally high level of DT-diaphorase. There was no difference with radicicol in the isogenic transfected BE cell line pair.

The correlation seen between expression of DT-diaphorase activity and sensitivity to 17AAG but not to geldanamycin or radicicol shows that the effect is not generic across all Hsp90 inhibitors or, indeed, across all benzoquinone ansamycins. The precise mechanism by which high levels of DT-diaphorase in tumor cells result in sensitivity to 17AAG is not clear. The observation that DT-diaphorase activity affects tumor cell sensitivity to 17AAG but not to geldanamycin or 17-amino,17-demethoxygeldanamycin is not explicable in terms of their respective behavior as substrates for the purified human enzyme. Although we have demonstrated that 17AAG is a reasonable substrate for human DT-diaphorase, it was not appreciably better than geldanamycin or 17-amino,17-demethoxygeldanamycin, particularly at more relevant drug concentrations. Only at the markedly suprapharmacologic concentrations of 50 and 100 μ M was 17AAG reduced at a statistically significantly faster rate than geldanamycin. For 17-amino,17-demethoxygeldanamycin, there was no appreciable difference in rate compared with geldanamycin.

Given the close structural similarity of 17AAG, 17-amino,17-demethoxygeldanamycin, and geldanamycin (Fig. 1), it is clear that it is the allyl substitution on the amino group at position 17 that is responsible for the DT-diaphorase effect. Preliminary results with a range of 17AAG analogues are consistent with this observation. We hypothesize that the behavior of the reduction product of 17AAG must differ from the reduction products derived from geldanamycin analogues with other substituents.

The xenograft experiment confirmed that DT-diaphorase-transfected BE-F397 cells were more sensitive than BE vector control cells in a solid tumor *in vivo*. The naturally high DT-diaphorase-containing HT29 xenograft was also more sensitive than the BE vector control xenograft. Dose-response data were not generated in these experiments. However, it seems likely that the differences seen in the *in vivo* xenografts were not as large as those observed in the same lines *in vitro*. One factor that would tend to decrease the contribution of DT-diaphorase levels in the xenograft experiments is the metabolism of 17AAG to the 17-amino derivative, which is the major metabolite in the mouse (13). This could be important because we show in this article that sensitivity to the 17-amino metabolite is not affected by DT-diaphorase. Formation of the 17-amino metabolite is catalyzed by cytochrome P450, specifically CYP3A4 in human microsomes (13). Thus, we propose that the sensitivity of a given patient's tumor to 17AAG may be affected by the balance between DT-diaphorase and CYP3A4 metabolism. Consequently, we urge that both enzymes (or surrogates thereof) be monitored in the clinical studies that are now under way with 17 AAG.

We determined that 17AAG was operating through the Hsp90 protein to stimulate degradation of the oncogenic client proteins Raf-1 and mutant p53 by use of 17AAG at equitoxic and equimolar concentrations and cells expressing high and low levels of DT-diaphorase. The depletion of client proteins reported previously for both 17AAG and geldanamycin (4-6,9) was seen in cells expressing high and low levels of DT-diaphorase. At equitoxic concentrations of 17AAG or geldanamycin (5 \times and 10 \times IC₅₀) in the isogenic BE cell lines after 6 hours and, especially, after 24 hours of drug exposure, there was a similar and marked reduction in Raf-1 and mutant p53 proteins. At the fixed

concentrations of 0.15 or 0.3 μM 17AAG, which inhibited growth of wild-type NQO1-transfected cells but not BE vector control cells, there was no reduction in Raf-1 or p53 protein in cells with low levels of DT-diaphorase, whereas depletion was seen in the cells with high levels of DT-diaphorase that did respond to these concentrations. Thus, target activity was maintained in the presence of the respective active concentrations of 17AAG, independent of the expression of DT-diaphorase. This rules out the possibility that different target mechanisms operate in cells expressing low and high levels of DT-diaphorase. Rather, DT-diaphorase expression increases the potency of 17AAG via client protein depletion.

In contrast to effects reported in melanoma xenografts after administration of 17AAG (11), no difference in the levels of Hsp90 was observed in our experiments. Hsp70 levels, however, were increased, consistent with the removal of Hsp90-induced transcriptional repression of Hsp70 when Hsp90 is inhibited (38). Again, this effect was seen at equitoxic concentrations of 17AAG in both high and low DT-diaphorase lines, consistent with retention of the Hsp90-binding mechanism.

The high constitutive expression of p53 in BE cells suggests a mutant p53 genotype. Effects on mutant p53 were consistent with cell cycle effects of geldanamycin reported in cell lines expressing wild-type or mutant p53 (39). In our own studies on the A2780 human ovarian carcinoma cell line (wild-type for p53) and a subline stably transfected with the viral p53-inactivating gene HPVE6 (40), we found no difference in sensitivity to geldanamycin or 17AAG. Overall, the results indicate that p53 status is unlikely to influence sensitivity to 17AAG.

In summary, although uncertainties remain regarding the precise mechanism involved, our results clearly show that expression of DT-diaphorase can influence a tumor's sensitivity to 17AAG. It is also possible that NQO1 expression could affect toxicity of 17AAG toward normal tissues. There are obvious implications for the clinical evaluation of 17AAG as an anticancer agent because 5%–20% of the population (depending on ethnicity) is homozygous for the genetic polymorphism used in this study, the DT-diaphorase-disabling point mutation in the NQO1 gene present in the BE colon cell line (41). In addition, the expression of DT-diaphorase in human tumors is very variable (25,26), as it is in the cell lines studied herein and elsewhere (22–24). We suggest that, in addition to measuring degradation of oncogenic client proteins and/or an increase in Hsp70 after treatment with 17AAG as potential markers of activity and therapeutic response, NQO1/DT-diaphorase genotype, CYP3A4 status, and also tumor DT-diaphorase levels should be determined. In particular, we propose that these measurements may provide useful indicators of efficacy and/or toxicity and should be considered for the phase I clinical trials of 17AAG that have recently begun under the auspices of the NCI and CRC.

REFERENCES

- Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. *Proc Natl Acad Sci U S A* 1994;91:8324–8.
- Stebbins CE, Russo AA, Schneider C, Rosen N, Hartl FU, Pavletich NP. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell* 1997;89:239–50.
- Prodromou C, Roe SM, O'Brien RO, Ladbury JE, Piper PW, Pearl LH. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* 1997;90:65–75.
- An WG, Schnur RC, Neckers L, Blagosklonny MV. Depletion of p185^{erbB2}, Raf-1 and mutant p53 proteins by geldanamycin derivatives correlates with antiproliferative activity. *Cancer Chemother Pharmacol* 1997;40:60–4.
- Dasgupta G, Momand J. Geldanamycin prevents nuclear translocation of mutant p53. *Exp Cell Res* 1997;237:29–37.
- Whitesell L, Surphin PD, Pulcini EJ, Martinez JD, Cook PH. The physical association of multiple molecular chaperone proteins with mutant p53 is altered by geldanamycin, an hsp90-binding agent. *Mol Cell Biol* 1998;18:1517–24.
- Supko JG, Hickman RL, Grever MR, Malspeis L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. *Cancer Chemother Pharmacol* 1995;36:305–15.
- Schnur RC, Corman ML, Gallaschun RJ, Cooper BA, Dee MF, Doty JL, et al. erbB-2 oncogene inhibition by geldanamycin derivatives: synthesis, mechanism of action, and structure-activity relationships. *J Med Chem* 1995;38:3813–20.
- Schulte TW, Neckers LM. The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. *Cancer Chemother Pharmacol* 1998;42:273–9.
- Page J, Heath J, Fulton R, Yalkowsky E, Tabibi E, Tomaszewski J, et al. Comparison of geldanamycin (NSC-122750) and 17-allylamino-geldanamycin (NSC-330507D) toxicity in rats [abstract]. *Proc Am Assoc Cancer Res* 1997;38:A2067.
- Burger AM, Fiebig HH, Newman DJ, Camalier RF, Sausville EA. Antitumor activity of 17-(allylamino)-17-demethoxygeldanamycin (NSC 330507) in melanoma xenografts is associated with decline in Hsp 90 protein expression [abstract]. *Annals Oncol* 1998;9(Suppl 2):abstract 304.
- Eiseman JL, Sentz DL, Zuhowski EG, Ramsland TS, Rosen DM, Reyna SP, et al. Plasma pharmacokinetics and tissue distribution of 17-allylamino-geldanamycin (NSC 330507), a prodrug for geldanamycin, in CD₂F₁ mice and Fisher 344 rats [abstract]. *Proc Am Assoc Cancer Res* 1997;38:A2063.
- Egorin MJ, Rosen DM, Wolff JH, Callery PS, Musser SM, Eiseman JL. Metabolism of 17-(allylamino)-17-demethoxygeldanamycin (NSC 330507) by murine and human hepatic preparations. *Cancer Res* 1998;58:2385–96.
- Sharma SV, Agatsuma T, Nakano H. Targeting of the protein chaperone, HSP90, by the transformation suppressing agent, radicicol. *Carcinogenesis* 1998;16:2639–45.
- Soga S, Kozawa T, Narumi H, Akinaga S, Irie K, Matsumoto K, et al. Radicicol leads to selective depletion of Raf kinase and disrupts K-Ras-activated aberrant signaling pathway. *J Biol Chem* 1998;273:822–8.
- Schulte TW, Akinaga S, Soga S, Sullivan W, Stensgard B, Toft D, et al. Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. *Cell Stress Chaperones* 1998;3:100–8.
- Roe SM, Prodromou C, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. *J Med Chem* 1999;42:260–6.
- Ernster L. DT-diaphorase: a historical review. *Chemica Scripta* 1987;27A:1–13.
- Workman P. Enzyme-directed bioreductive drug development revisited: a commentary on recent progress and future prospects with emphasis on quinone anticancer agents and quinone metabolising enzymes, particularly DT-diaphorase. *Oncol Res* 1994;6:461–75.
- Riley RJ, Workman P. DT-diaphorase and cancer chemotherapy. *Biochem Pharmacol* 1992;43:1657–69.
- Stratford IJ, Workman P. Bioreductive drugs into the next millennium. *Anticancer Drug Des* 1998;13:519–28.
- Robertson N, Stratford IJ, Houlbrook S, Carmichael J, Adams GE. The sensitivity of human tumor cells to quinone bioreductive drugs: what role for DT-diaphorase? *Biochem Pharmacol* 1992;44:409–12.
- Fitzsimmons SA, Workman P, Griever M, Paull K, Camalier R, Lewis AD. Reductase enzyme expression across the National Cancer Institute Tumor Cell Line Panel: correlation with sensitivity to mitomycin C and EO9. *J Natl Cancer Inst* 1996;88:259–69.
- Ross D, Beall HD, Siegel D, Traver RD, Gustafson DL. Enzymology of bioreductive drug activation. *Br J Cancer Suppl* 1996;27:S1–8.
- Schlager JJ, Powis G. Cytosolic NAD(P)H: (quinone acceptor) oxidoreduc-

- tase in human normal and tumor tissue: effects of cigarette smoking and alcohol. *Int J Cancer* 1990;45:403-9.
- (26) Marin A, Lopez de Cerain A, Hamilto, Lewis AD, Martinez-Penuela JM, Idoate MA, et al. DT-diaphorase and cytochrome b₅ reductase in human lung and breast tumours. *Br J Cancer* 1997;76:923-9.
 - (27) Brunton VG, Steele G, Lewis AD, Workman P. Geldanamycin-induced cytotoxicity in human colon-cancer cell lines: evidence against the involvement of c-Src or DT-diaphorase. *Cancer Chemother Pharmacol* 1998;41:117-22.
 - (28) Monks A, Scudiero DA, Johnson GS, Paull KD, Sausville EA. The NCI anti-cancer drug screen: a smart screen to identify effectors of novel targets. *Anticancer Drug Des* 1997;12:533-41.
 - (29) Traver RD, Horikoshi T, Danenberg KD, Stadlbauer THW, Danenberg PV, Ross D, et al. NAD(P)H: quinone oxidoreductase gene expression in human colon carcinoma cells: characterization of a mutation which modulates DT-diaphorase activity and mitomycin sensitivity. *Cancer Res* 1992;52:797-802.
 - (30) Holford J, Sharp SY, Murrer BA, Abrams M, Kelland LR. *In vitro* circumvention of cisplatin resistance by the novel sterically hindered platinum complex AMD473. *Br J Cancer* 1998;77:366-73.
 - (31) Sharp SY, Rowlands MG, Jarman M, Kelland LR. Effects of a new anti-oestrogen, idoxifene, on cisplatin- and doxorubicin-sensitive and -resistant human ovarian carcinoma cell lines. *Br J Cancer* 1994;70:409-14.
 - (32) Sharp SY, Smith V, Hobbs S, Kelland LR. Lack of a role for MRP1 in platinum drug resistance in human ovarian cancer cell lines. *Br J Cancer* 1998;78:175-80.
 - (33) Hobbs S, Jitrapakdee S, Wallace JC. Development of a bicistronic vector driven by the human polypeptide chain elongation factor 1 α promoter for creation of stable mammalian cell lines that express very high levels of recombinant proteins. *Biochem Biophys Res Commun* 1998;252:368-72.
 - (34) Walton MI, Smith PJ, Workman P. The role of NAD(P)H: quinone reductase (EC 1.6.99.2, DT-diaphorase) in the reductive bioactivation of the novel indoloquinone antitumor agent EO9. *Cancer Commun* 1991;3:199-206.
 - (35) Beall HD, Liu Y, Siegel D, Bolton EM, Gibson NW, Ross D. Role of NAD(P)H: quinone oxidoreductase (DT-diaphorase) in cytotoxicity and induction of DNA damage by streptonigrin. *Biochem Pharmacol* 1996;51:645-52.
 - (36) Workman P, Twentyman P, Balkwill F, Balmain A, Chaplin D, Double J, et al. United Kingdom Co-ordinating Committee on Cancer Research guidelines for the welfare of animals in experimental neoplasia. 2nd ed. *Br J Cancer* 1998;77:1-10.
 - (37) Moscow JA, Connolly T, Myers TG, Cheng CC, Paull K, Cowan KH. Reduced folate carrier gene (RFC1) expression and anti-folate resistance in transfected and non-selected cell lines. *Int J Cancer* 1997;72:184-90.
 - (38) Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R. Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell* 1998;94:471-80.
 - (39) McIlwrath AJ, Brunton VG, Brown R. Cell-cycle arrests and p53 accumulation induced by geldanamycin in human ovarian tumour cells. *Cancer Chemother Pharmacol* 1996;37:423-8.
 - (40) Walton MI, Pestell KE, Valenti MR, Brunton LA, Kelland LR, Workman P. A comparison of *in vitro* and *in vivo* techniques for assessing chemosensitivity in a genetically engineered cell line [abstract]. *Proc Am Assoc Cancer Res* 1999;40:407.
 - (41) Kelsey KT, Ross D, Traver RD, Christiani DC, Zuo ZF, Spitz MR, et al. Ethnic variation in the prevalence of a common NAD(P)H quinone oxidoreductase polymorphism and its implications for anti-cancer chemotherapy. *Br J Cancer* 1997;76:852-4.

NOTES

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